vitrifying the dehydrated specimen, by cooling to a refrigeration or higher storage temperature.

B3 7. (Twice Amended) The method of claim 1, wherein the total concentration of non-permeating co-solute is between 0.1 and 0.7 mol/l.

9. (Twice Amended) The method of claim 1, wherein dehydrating the specimen is performed in two or more stages of contacting the specimen with increasingly higher

B4 concentrations of the permeating cryoprotectant and the co-solute.

10. (Twice Amended) The method of claim 1, wherein dehydrating the specimen is performed by simultaneously increasing concentrations of both permeating cryoprotectant and the co-solute from an initial concentration to a final concentration according to a desired profile.

12. (Twice Amended) The method of claim 1, further comprising rehydrating the

B5 specimen by contacting the vitrified specimen with a rehydration solution comprising a non-permeating rehydration co-solute which effectively decreases the chemical potential of a permeating rehydration cryoprotectant.

25. (Amended) The method of claim 1, wherein the non-permeating co-solute is selected

B6 from the group consisting of an aldose monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, aidonic acid, uronic acid, aldaric acid, and a disaccharide.

26. (Amended) The method of claim 12, wherein the non-permeating rehydration co-solute is selected from the group consisting of an aldose monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, aidonic acid, uronic acid, aldaric acid, and a disaccharide.

REMARKS

Claims 1, 7, 9, 10, and 12 have been amended. Support for the amendments is found in the existing claims and the specification. Accordingly, the amendments do not constitute the addition of new matter. Applicant respectfully requests the entry of the amendments and reconsideration of the application in view of the amendments and the following remarks. Applicant believes that entry of the present amendment will, at least, reduce the number of issues for appeal.

The specific changes to the specification and the amended claims are shown on a separate set of pages attaches hereto and entitled **VERSION WITH MARKINGS TO SHOW**

CHANGES MADE, which follows the signature page of this Amendment. On this set of pages, insertions are underlined and deletions are struck through.

Information disclosure statement

A supplemental Information Disclosure Statement will be filed with complete citations for references 1, 4, 5, and 7.

Rejections under 35 U.S.C. §112, second paragraph

The Examiner asserts that it is uncertain what constitutes "a refrigeration temperature" and provides a definition from a medical dictionary which states that refrigeration is "the act of lowering the temperature of a body by conducting away its heat to a surrounding cooler substance." However, Applicant's use of the word is meant to convey the ordinary meaning of the word found in common speech. This meaning is represented by Webster's II New Riverside Dictionary, revised edition, a copy of which is submitted as Attachment A. By this definition, refrigeration (refrigerate) is defined as "To chill (a substance) in a refrigerator." This is the meaning of "refrigeration" in the context of Applicant's claimed invention. That is, the use of mechanical means to cool a sample, such as a refrigerator, as opposed to chemical means such as liquid nitrogen which is the method of the prior art.

It is clear from a reading of the specification as a whole that Applicant's use of the word "refrigeration" corresponds to the meaning provided by Webster's New Riverside Dictionary. For example, at page 1, lines 20-27, Applicant states, "Conventionally, cryopreservation by vitrification of single cell...and multicellular specimens provide for storage of cryopreserved samples at -196°C in liquid N_2. However, there is currently a need for reliable methods for long-term shelf preservation at refrigeration or higher temperatures." That is, Applicant's intent is clearly to avoid the use of very low temperatures such as those produced by liquid nitrogen. Again at page 10, lines 23-25 of the present specification, Applicant recites that "the invention provides a method for shelf preservation of cells and multicellular specimens at refrigeration or higher temperatures." Thus, it is clear from the plain dictionary definition of the word "refrigeration" and from a reading of the specification as a whole, that Applicant's invention is drawn to the use of temperatures that are obtainable by the use of a refrigerator, which would be from about 10°C to about -70°C.

The Examiner further asserts that the term "cooling" is unclear as it is a relative term with no reference point. However, it is clear from the specification that the "cooling" occurs from ambient temperatures to temperatures that are obtainable by use of a refrigerator. See, for example, present specification, page 13, line 36 to page 14, line 1 which states that "The method of the present invention encompasses dehydration of specimens, cooling samples to storage temperature, warming of the samples to ambient temperature...". Thus, it is clear that by a careful reading of the specification, the temperature range for the claimed method is from ambient temperatures, about 20°C, to refrigeration temperatures, about -70°C. In view of Applicant's arguments, it is respectfully requested that the Examiner reconsider and withdraw the grounds of rejection for claim 1.

The grounds of rejection for claims 13, 25, and 26 are believed to be rendered moot by Applicant's amendment of claims 12, 25, and 26.

New matter

Claims 1, 4-10, 12-17, 25, and 26 are rejected under 35 112, first paragraph as containing new matter. This ground of rejection is believed to be overcome by Applicant's amendment.

Rejections under 35 U.S.C. §102

Claims 1, 4-7, 16, and 25 were rejected under §102(b) as being anticipated by Titterington et al. This reference teaches immersion of specimens in liquid nitrogen (-196° C) in the presence of sucrose (a non-permeating co-solute), glycerol (a permeating cryoprotectant) and Percoll (a non-permeating cryoprotectant). However, the disclosure of Titterington et al. is limited to rapid freezing to –196° C. Titterington et al. does not teach dehydrating a specimen in a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant, and then vitrifying the dehydrated specimen by cooling to a refrigerated or higher storage temperature, as recited in present Claim 1. As set forth above in response to the rejection under 35 U.S.C. §112, second paragraph, Applicant's claims encompass refrigeration temperatures, that is, temperatures obtainable by the use of a refrigerator. Consequently, Applicants' claims cannot be anticipated by a teaching that includes freezing to -196°C.

Accordingly, Applicant respectfully requests withdrawal of the §102 rejection of Claims 1, 4-7, 16, and 25 based on Titterington et al.

Claims 1-6, 9, 10, 16, 18-24 and 25 remain rejected under 35 U.S.C. § 102 as being anticipated by Rall et al. Claims 18-24 have been canceled.

Rall et al. discloses progressive or step-wise cooling of embryos down to –196° C (liquid nitrogen). Applicant's claims are drawn to vitrification at refrigeration temperatures. See the discussion above regarding Applicant's definition of refrigeration. Rall et al. cannot anticipate Applicant's claims as the disclosure of Rall et al. is drawn to the use of much lower temperatures.

In view of Applicant's arguments, withdrawal of this ground of rejection is respectfully requested.

Claims 1, 4-7, 9, 12-16, 25 and 26 remain/are rejected under 35 U.S.C. § 102 (b) as anticipated by U.S. Patent No. 5,364,756.

The '756 patent teaches the use of very low temperatures, e.g., -196°C (see col. 17 lines 15-39 and Example 4 which teaches temperatures of -160°C. Again, Applicant's claimed method is not drawn to the use of such low temperatures. Applicant's method is directed to the use of temperatures which can be achieved with the use of a refrigerator. Temperatures of -160°C to -196°C cannot be achieved by a refrigerator. Consequently, Applicant's claims are not anticipated by the '756 reference.

Withdrawal of this ground of rejection is respectfully requested.

Claims 1, 4-7, 9, 10, 12-17, 25, and 26 remain rejected under §102(e) as anticipated by U.S. Patent No. 5,800,978 to Goodrich. As noted by the Examiner, Goodrich discloses numerous cryopreservation solutions comprising permeating cryoprotectants, non-permeating co-solutes and non-permeating cryoprotectants. Goodrich also teaches freezing cells in these cryopreservation solutions, and in some cases (See e.g., Example 6), subsequent dehydration by sublimation. However, Goodrich does not disclose vitrification as claimed by Applicant. Goodrich et al disclose a method which "provides a multi-component aqueous cryopreservative system(s) that at appropriate temperatures form partially crystalline mixtures of water ice with interspersed regions of a separate amorphous glass phase" (col. 4, lines 15-18). Also, in contradistinction to Applicant's claimed method, Goodrich teaches against the use of DMSO

(col. 3, lines 25-26). Consequently, the disclosure of Goodrich is drawn to a different method than the method claimed by Applicant.

The Examiner argues that Goodrich inherently teaches the claimed method because Goodrich teaches Applicant's three classes of compounds (Paper No. 8, page 8, lines 1-2). However, as noted above, the compounds taught by Goodrich are not synonymous with the compounds of the presently claimed invention. Goodrich teaches away from the use of DMSO. Furthermore, Goodrich does not teach a non-permeating co-solute. To the extent that the compounds used by Goodrich may coincidentally fall into the categories taught by Applicant, it is noted that the specific combinations taught by Goodrich do not correspond to the specific components taught by Applicant. For example, the Examiner cites buffer No.8 of Table 2, maintaining that the glucose component corresponds to the permeant component (Paper No. 8, page 7, lines 16-18). However, glucose is not one of the compounds listed in Applicant's claim 4 as a permeant component.

Consequently, Goodrich does not teach all of the elements of Applicant's claimed invention and the rejection under 35 U.S.C. § 102 may be properly withdrawn.

Rejections under 35 U.S.C. §103

Claims 1, 4-10, 12-16, 25-26 are/remain rejected under 35 U.S.C. § 103(a) as unpatentable over U.S. 5,364,756 in view of 5,217,860 taken with U.S. 4,865,871 or Rall et al. and U.S. 5,879,876.

The primary reference fails for the reasons given above in the response to the rejection under 35 U.S.C. § 102. The '756 patent teaches the use of very low temperatures, e.g., -196°C (see col. 17 lines 15-39 and Example 4 which teaches temperatures of -160°C). Again, Applicant's claimed method is not drawn to the use of such low temperatures. Applicant's method is directed to the use of temperatures which can be achieved with the use of a refrigerator. Temperatures of -160°C to -196°C cannot be achieved by a refrigerator.

None of the cited references alone or in combination teach or suggest vitrifying a dehydrated specimen by cooling to a refrigeration or higher storage temperature as recited in claim 1. The '860 disclosure teaches temperatures of -135 °C (col. 24, line 37). The '871 disclosure teaches the use of temperatures of -196°C (col. 9, lines 40-42). The '876 disclosure

teaches "ultra low temperature" (col. 15, lines 7). None of the cited references teach or suggest vitrification at higher temperatures.

Since all claims depend from claim 1, which is neither taught nor suggested by the cited references as discussed above, the invention defined in claims 4-10, 12-16, 25 and 26 is also patentably distinguished from the references, alone or in combination. Applicant respectfully requests the withdrawal of the rejection.

CONCLUSIONS

In view of the foregoing amendments and remarks, the present application is submitted as in condition for allowance, and such action is earnestly solicited. If any matters should remain, the Examiner is invited to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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Dated: April 4, 2001

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (Twice Amended) A method for preserving a cell or tissue specimen comprising:

equilibrating and thereby dehydrating the specimen [with] in a vitrification solution comprising a permeating cryoprotectant, a non-permeating co-solute and a non-permeating polymeric cryoprotectant, wherein the non-permeating co-solute effectively decreases the chemical potential of the permeating cryoprotectant thereby limiting the amount of the permeating cryoprotectant which permeates into the specimen; and

vitrifying the dehydrated specimen, ~~without freezing,~~ by cooling to a refrigeration or higher storage temperature.

4. (Amended) The method of claim 1, wherein the permeating cryoprotectant is selected from the group consisting of dimethylsulfoxide, ethylene glycol, propylene glycol and glycerol.

5. (Amended) The method of claim 1, wherein the non-permeating polymeric cryoprotectant is selected from the group consisting of dextrans, starches, polyethylene glycol, polyvinylpyrrolidone, FICOLL and peptides.

6. (Amended) The method of claim 1, wherein the non-permeating co-solute is selected from the group consisting of an amino acid, an amino acid derivative.

7. (Twice Amended) The method of claim 1, wherein the total concentration of non-permeating co-solute is between ~~about~~ 0.1 and 0.7 mol/l.

9. (Twice Amended) The method of claim 1, wherein ~~equilibrating and thereby~~ dehydrating the specimen is performed in two or more stages of contacting the specimen with increasingly higher concentrations of the permeating cryoprotectant and the co-solute.

10. (Twice Amended) The method of claim 1, wherein ~~equilibrating and thereby~~ dehydrating the specimen is performed by simultaneously increasing concentrations of both permeating cryoprotectant and the co-solute from an initial concentration to a final concentration according to a desired profile.

12. (Twice Amended) The method of claim 1, further comprising rehydrating the specimen by contacting the vitrified specimen with a rehydration solution comprising a non-permeating rehydration co-solute which effectively decreases the chemical potential of ~~the~~ a permeating rehydration cryoprotectant.

25. (Amended) The method of claim 1, wherein the non-permeating co-solute is selected from the group consisting of an aldose monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, aidonic acid, uronic acid, aldaric acid, and a disaccharide ~~and a polysaccharide~~.

26. (Amended) The method of claim 12, wherein the non-permeating rehydration co-solute is selected from the group consisting of an aldose monosaccharide, a ketose monosaccharide, an amino sugar, an alditol, an inositol, aidonic acid, uronic acid, aldaric acid, and a disaccharide ~~and a polysaccharide~~.

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